

Overview of Molecular Methods in Immunohematology

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Overview

- **Limitations of hemagglutination**
- **Need for larger inventories of antigen-negative donor RBC components**
- **Value of microarray technology**
- **Value of DNA testing for fetuses, patients and donors**
- **Limitations of DNA testing**
- **Other considerations**

Hemagglutination: Limitations

- It gives only an indirect indication of a fetus at risk of hemolytic disease of the newborn
- It is difficult to phenotype a recently multi-transfused patient
- It can be difficult to phenotype RBCs coated with IgG
- A relatively small number of donors can be typed for a relatively small number of antigens, which limits antigen-neg inventory
- Cannot reliably determine zygosity
- It is a subjective test

Stroke Prevention Trials

STOP I - stopped early (after 18 months)

- **Continuous transfusion prevents strokes**

STOP II - 6 year trial after aborted 2 years

- **Of 41pts selected to discontinue transfusion**
 - 14 reverted to high risk transcranial Doppler ultra-sound images
 - 2 suffered strokes
- **Of 38 patients who continued to receive transfusions**
 - None had strokes
 - None reverted to high-risk state

**Cannot withhold transfusions, because of risks
decisions must be on a case-by-case basis**

Blood Transfusion Support for SCD Patients

If we are to transfuse these patients effectively, we must find more effective ways to reduce risk of:

- Transfusion reactions**
- Hyper-hemolysis syndrome**
- Alloimmunization**

For the first time in the history of blood transfusion, microarray technology will make it feasible to contemplate precisely matching the antigen-negative status of a donor to that of a patient destined to receive chronic transfusions

Hemagglutination for Minor Antigens

- **Labor-intensive manual testing**
- **Labor-intensive manual data entry**
- **Source material is expensive and diminishing**
- **Cost of commercial reagents (FDA-approved) is escalating (screening versus confirmation)**
- **Many antibodies are not FDA-approved and are characterized (often partially) by the user**
- **Some antibodies are limited in volume, weakly reactive, or not available**

Blood Groups and DNA Analysis

- **The genes encoding 28 of the 29 blood group systems have been sequenced (awaits gene for P1 antigen)**
- **The molecular bases of most blood group antigens and many phenotypes have been determined**
- **Thus, DNA assays (which use reagents that can readily be purchased and are not dependant upon human source material) can be used in the clinical laboratory to overcome limitations of hemagglutination**

Role of Molecular Techniques in Transfusion Medicine

- **Antigen typing**
 - To identify a fetus at risk for HDN
 - Patients: e.g., recently transfused and with +DAT
 - To screen for antigen-negative donors
 - Reagent RBCs (antibody screening and identification)
- **Determine zygosity, particularly *RHD***
- **Resolve discrepancies, e.g., A, B, D, C, c, e**

Role of Molecular Techniques in Transfusion Medicine (Cont.)

- **Identify molecular basis of unusual serological results**
- **One-step, automated, objective antibody detection and identification**
- **Transfected cells as immunogens**
- **Conversion of IgG Mabs to IgM**
- **Determine origin of engrafted leukocytes in a stem cell recipient**
- **Determine origin of lymphocytes in patient with graft-versus-host disease**

Fetus at Risk for HDN: DNA Analysis

- **Predict antigen type of fetus**
- **Sources of DNA**
 - Amniocytes
 - Fetal DNA in maternal plasma (deleted *RHD*)
- **Regardless of implicated antibody**
 - Always test for *RHD*, this preempts requests for D– RBCs for intrauterine transfusions (e.g., r'r')
- **Potential pitfalls include**
 - Contamination by maternal DNA
 - False negative results

DNA Typing of a Patient

- **Who has been recently transfused**
- **When RBCs are coated with IgG (+DAT)**
- **To distinguish allo from auto antibodies**
- **To detect weakly expressed antigens (e.g., Fy^b with Fy^x phenotype); where patient is unlikely to make antibodies to transfused antigen-positive RBCs**
- **To identify molecular basis of unusual serological results, especially Rh variants**

Recently Transfused Patients: Antigen Typing

- **“Best guess” of RBC phenotype is unreliable**
 - Mixed field hemagglutination
 - The number of RBC components transfused
 - Time since last transfusion
 - Estimated blood volume of patient
 - Prevalence of antigen
- **DNA testing**
 - Urine sediment, buccal smear, peripheral blood WBCs gave the same result, which was consistent with pre-transfusion hemagglutination tests
 - Donor WBCs did not interfere with PCR assays, at least in the way we perform them

DNA Analysis in a Patient Whose RBCs Have a Positive DAT

- **When direct agglutinating antibodies, or murine Mabs, are not available**
- **When antisera are weakly reactive**
- **When antigen is sensitive to the IgG removal treatment**
- **Distinguish allo from auto antibodies**

When to Use DNA to Antigen Type Donors

- **When antibody is weak or not available, e.g., Do^a/Do^b; Js^a/Js^b; V/VS; S/s!**
- **Mass screening to increase antigen-negative inventory and to find donors whose RBCs lack a high-prevalence antigen**
- **Reagent RBCs (antibody screening and identification)**
- **Resolution of discrepancies, e.g., A, B, D, C, e**
- **Detection of genes encoding weak antigens**

DNA Testing Donors for Reagent RBCs

DNA typing of donors for commercial RBC reagents is desirable and should be acceptable with appropriate disclaimers

- **To predict antigen type when suitable antibodies are not available, e.g., Do^a, Do^b, Js^a, V/VS**
- **To confirm homozygosity, especially S, D, Fy^a/Fy^b, Jk^a/Jk^b**
- **To define specific Knops antigen negativity versus CRI low copy number**

Desirable/recommended/mandated?

Testing for Antigen-Negative Donors

- Like all donors, antigen-negative donors must have their ABO and Rh type determined
- Due to complexity of genes encoding ABO and Rh blood groups, DNA analysis is not the method of choice for routine ABO and D determination
- Screening for rare donors by analysis of DNA is valuable for typing for 'minor' antigens and Rh variants
- Conserve precious antibodies to confirm DNA typing

Why Not Determine ABO and D by Testing DNA?

- The naturally-occurring anti-A and/or anti-B in the plasma of most people who lack the corresponding antigens provides a built-in check when performing ABO typing by hemagglutination
- Potent, well-standardized monoclonal reagents are available for ABO and D typing
- Hemagglutination is relatively simple and rapid
- Systems are in place to test and record, relatively efficiently, the ABO and D type of a donor
- There are few antigens and many alleles:
 - ABO: 2 antigens (A, B), 4 phenotypes (A, B, AB, O), >100 alleles known (probably more)
 - D: 1 antigen, >140 alleles known (probably more)
- D– C– E– RBCs are usually truly D–
- DNA testing is more time-consuming, more expensive, is prone to misinterpretation, and not an improvement over hemagglutination

DNA Analysis for ABO and Rh Types

- **Resolution of ABO and D discrepancies using DNA assays can be valuable to show a discrepancy is due to a genetic variant and not to technologist error or reagent failure and, thus, not an FDA reportable error**
- ***ABO* genotyping can be useful for distinguishing an acquired phenotype from an inherited one without laborious family studies**
- **Many Rh phenotypes cannot easily be defined by serological methods, either because suitable panels of monoclonal antibodies are not available or the antibodies are not available in needed strength or volume. DNA assays may be used to define some and precisely match the D and e antigen status of a donor to a recipient (especially those with SCD).**

Testing for Donors Lacking Do antigens

- RBC typing for Do^a, Do^b, Hy and Jo^a is notoriously difficult because the corresponding antibodies, although clinically significant, are often:
 - Weakly reactive
 - Available only in small volume
 - In sera containing other alloantibodies
- At NYBC, we use PCR-RFLP analysis to type:
 - Selected antigen-negative donors for *DOA* and *DOB*
 - Donors whose RBCs react weakly or do not react with anti-Gy^a for *HY* and *JO*. This has provided us with a larger inventory of valuable donors

Testing for Do polymorphisms by DNA analysis surpasses hemagglutination for antigen typing

DNA Typing: Low Prevalence Antigens

- NY patients often need Js(a–), V–, VS–, Go(a–), or DAK– RBC components. Providing the products is difficult because:
 - Patients make antibodies to these antigens in addition to several others, e.g., anti-C, -K, -Fy^a, -Jk^b
 - These immunogenic antigens are on up to 20% of RBCs from African American donors. Such RBCs have been transfused to patients in the STOP program. The patients have made antibodies to these “low prevalence” antigens.
 - These antigens are not on antibody screening RBCs
 - Appropriate antibodies are not available to screen for donors
 - The cross-match is not always reliable

DNA-based assays provide a tool to mass screen donors, thereby increasing the antigen-negative inventory and improving patient care

Problem Case

**Patient's serum contained anti-U,
-C, -E, -K, plus -VS, and -Js^a**

Of 95 eligible U– C– E– K– donors

4 VS– Js(a–)

27 VS+ and/or Js(a+)

64 VS and/or Js^a NT

**Anti-VS and anti-Js^a not available in
sufficient volume to pre-screen**

Medical Pitfalls

- **DNA and hemagglutination test results may not agree**
 - Recent transfusion
 - Stem cell transplantation
 - Natural chimerism
- **DNA results from somatic cells and from peripheral blood may not agree**
 - Stem cell transplantation
 - Natural chimerism

Obtain an accurate medical history

Limitations of DNA-based Assays

- A DNA-based result is not a phenotype
- Will detect grossly normal but unexpressed genes:
 - Would lead to a donor being falsely typed as antigen-positive
 - The loss of a valuable antigen-negative (null) donor
 - Would not jeopardize the safety of blood transfusion
- Confirmation by hemagglutination is recommended
 - With reagent antibody if available
 - And/or cross-matching by a method optimal for the antibody/antigen in question
- Not all polymorphisms can be analyzed
 - if a large # of alleles encode 1 phenotype (ABO, Rh, nulls), or molecular basis is not yet known (Vel, Lan, Jr^a)
- There is a high probability that not all alleles in all ethnic populations are known

Institutional Review Board (IRB): Considerations

- **Clinical or research**
- **Existing or specific collection**
- **Unlinked or linked**
- **No risk or at risk**

Exempt, Expedited, Full Review

Typing by DNA Assays: Concern and Consent

- Donor consent may or may not be needed. This will depend on the wording of Donor Registration Forms with regard to how the testing for blood groups antigens will be performed.
- There is much concern that such typing will reveal unwanted information about a donor. However, the assays are not considered to be “genotyping” but rather are a procedure to antigen type by DNA analysis.
- According to NYS, as this DNA testing is not used to identify or diagnose a genetic disease, informed consent is not required

Antigen Typing by MicroArray: Cost Concerns

- **Direct savings**
 - Does not use precious, expensive reagents
 - Manual testing and data entry eliminated
 - Less skilled labor required for testing using kits
 - Reduced complex testing to detect alloantibodies in AIHA
- **Possible savings**
 - Less likely to import blood from overseas
 - Fewer transfusion reactions to treat
 - Less likely to withhold transfusion, thereby reducing number of days in hospital
 - No need to give antenatal Rhlg when fetus is Rh-negative

How much will the manufacturers charge?

Expense of investigating discrepancies?

Can we reduce charges for antigen-negative RBCs?

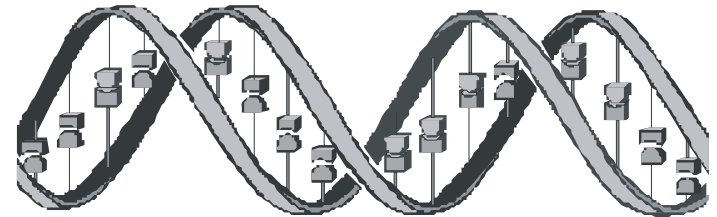
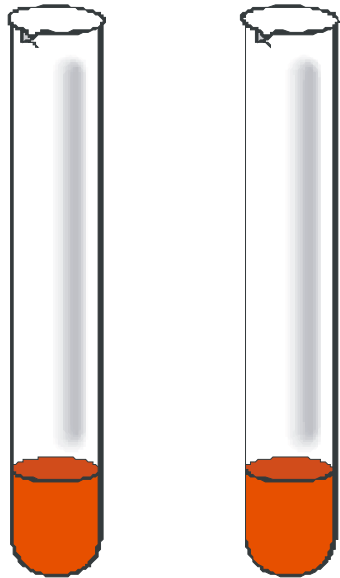
Possible Use of Phenotypically Matched Blood

- To match antigen profile in chronically transfused responders, especially those with SCD disease
 - With unusual Rh phenotypes (hr^{B-} , hr^{S-} , etc.)
 - Antigens for which there is no antibody (V/VS , Go^a , DAK , Js^a , Do^a , Do^b)
- To match for Jk^a and Jk^b if patient has been exposed, to prevent transfusion reactions and deaths due to anti- Jk^a or anti- Jk^b (FDA, SHOT)
- To match for D_{el} and D_{weak}
 - Transfusion weak D RBCs to patients with weak D
 - Save true O– blood for D– patients (use C–, E–)
- For patients with antibodies to high prevalence antigens
- For patients with AIHA to eliminate labor intensive procedures to ensure no underlying antibodies

Other considerations

- Establish extent of testing alleles for each antigen, e.g., GATA & nt 265 with *FY*
- Type donor once; IF we had a simple, inexpensive way to positively identify donor at subsequent donations
- Use result without confirmation by hemagglutination (unlikely to harm the patient)
- Automated DNA preparation and positive sample ID from beginning to end
- Natural chimeras
 - Rare?
 - DNA from WBCs will likely match RBC type

Tools Available



Hemagglutination ↔

Molecular Biology